Serum Dioxin Concentrations and Endometriosis: A Cohort Study in Seveso, Italy

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Dioxin, a ubiquitous contaminant of industrial combustion processes including medical waste incineration, has been implicated in the etiology of endometriosis in animals. We sought to determine whether dioxin exposure is associated with endometriosis in humans. We conducted a population-based historical cohort study 20 years after the 1976 factory explosion in Seveso, Italy, which resulted in the highest known population exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Participants were 601 female residents of the Seveso area who were ≤ 30 years old in 1976 and had adequate stored sera. Endometriosis disease status was defined by pelvic surgery, current transvaginal ultrasound, pelvic examination, and interview (for history of infertility and pelvic pain). "Cases" were women who had surgically confirmed disease or an ultrasound consistent with endometriosis. "Nondiseased" women had surgery with no evidence of endometriosis or no signs or symptoms. Other women had uncertain status. To assess TCDD exposure, individual levels of TCDD were measured in stored sera collected soon after the accident. We identified 19 women with endometriosis and 277 nondiseased women. The relative risk ratios (RRRs) for women with serum TCDD levels of 20.1–100 ppt and >100 ppt were 1.2 [90% confidence interval (CI) = 0.3–4.5] and 2.1 (90% CI = 0.5–8.0), respectively, relative to women with TCDD levels ≤ 20 ppt. Tests for trend using the above exposure categories and continuous log TCDD were nonsignificant. In conclusion, we report a doubled, nonsignificant risk for endometriosis among women with serum TCDD levels of 100 ppt or higher, but no clear dose response. Unavoidable disease misclassification in a population-based study may have led to an underestimate of the true risk of endometriosis. *Key words:* dioxin, endometriosis, environmental exposures, epidemiology. *Environ Health Perspect* 110:629–634 (2002). [Online 15 May 2002]
http://ehpnet1.niehs.nih.gov/docs/2002/110p629-634eskenazi/abstract.html

The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin), the most toxic halogenated aromatic hydrocarbon (*1*), is a ubiquitous contaminant of various industrial and combustion processes, including medical waste incineration. Dioxin is classified as a known human carcinogen (*1*), and concern about the reproductive toxicity of dioxin has been growing (*2*). Partially because of this concern, the U.S. Environmental Protection Agency and the World Health Organization have conducted a reassessment of dioxin, including human health consequences (*3,4*).

In the past decade, several animal studies have suggested that prenatal and postnatal exposure to dioxin and dioxin-like chemicals may profoundly affect the reproductive systems of both male and female animals perhaps via endocrine disruption (*2*). Some articles have concluded that dioxin-like compounds may be responsible for the failure of certain animal species to reproduce (*5*) and for a decrease in human sperm count (*6*). Another publication has noted high frequencies of endometriosis among infertile women living in Belgium, where breast milk concentrations of dioxin are among the highest in the world (*7*). The etiology of endometriosis is unknown (*8*). An association of dioxin with endometriosis is of public health significance because the estimated prevalence of endometriosis ranges from 1 to 10% in women of reproductive age (*8*) and because endometriosis is associated with significant costs for hospitalization and workdays lost (*8*).

Experimental animal evidence supports the association of endometriosis and exposure to dioxin-like chemicals. In 1993, Rier et al. (*9*) reported a dose–response relation between TCDD levels (5 and 25 ppt) in feed and the incidence and severity of endometriosis in 6- to 10-year-old adult rhesus monkeys, diagnosed a decade after dosing ceased. TCDD has also promoted the survival and growth of surgically induced endometrial implants in nonhuman primates (*10*) and in mice (*11–13*), but not in rats (*12*). More recent animal data suggest that endometriosis may also be associated with increased body burden of dioxin-like polychlorinated biphenyls (PCBs), particularly PCB 77 and PCB 126, and of total serum TCDD toxic equivalents (TEQ) (*14*).

The animal studies have stimulated a series of hospital-based endometriosis case–control studies in humans, with inconsistent results. A German study (*15*) found higher serum levels of three non-dioxin–like PCB congeners (PCBs 138, 153, 180) in cases than in controls. An Israeli study (*16*) reported low-levels of TCDD in 8 of the 44 cases (18%; range = 0.7–1.2 ppt) but in only 1 of the 35 (2%) controls (0.4 ppt), yielding a nonsignificant odds ratio of 7.6. Using the CALUX assay, a Belgian study (*17*) reported a high TEQ (> 100 pg/g) in 6 of 42 cases (14%) but in only 1 of 27 controls (4%), for a nonsignificant crude odds ratio of 4.3 [95% confidence interval (CI) = 0.49–43.6). Studies in the United States (*18*) and Canada (*19*) found no association between human levels of selected environmental chemicals and endometriosis. The U.S. study measured TCDD and 21 other polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and PCB congeners, but had a small sample size (15 cases and geographically matched controls). However, the Canadian study, the largest study of all (86 cases and 70 controls), found no differences between levels in cases and controls when measuring 14 noncoplanar and coplanar PCB

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We gratefully acknowledge L. Bonsignore (Desio Hospital) for examining women at the Hospital of Desio, and members of the Scientific Advisory Board: D. Baird, L. Birnbaum, and D. Cramer. We would especially like to thank the women who participated in this study.

This study was supported by grants R01 ES07171 and F06 TW02075-01 from the National Institutes of Health, R82471 from the U.S. Environmental Protection Agency, EA-M1977 from the Endometriosis Association, 2P30-ESO01896-17 from the National Institute of Environmental Health Sciences, and 2896 from Regione Lombardia and Fondazione Lombardia Ambiente, Milan, Italy.

Received 13 April 2001; accepted 27 December 2001.

congeners (including the same PCBs measured in the positive German study) and 11 chlorinated pesticides; they did not measure TCDD or other dioxins or furans. No studies specifically measured the dioxin-like PCB congeners found to be related to endometriosis in Rier et al.'s study (*14*). In general, small sample size and/or a failure to evaluate exposure to dioxins or other dioxin-like compounds limited most of these studies.

If a relationship exists between dioxin and endometriosis, it should be most apparent in a highly exposed population. In 1976, an explosion at a chemical factory near Seveso, Italy, resulted in the highest TCDD levels known in human residential populations (*20*). Bois and Eskenazi (*21*) conducted a toxicokinetic analysis of the TCDD levels in serum collected at the time of the explosion for 19 of the most heavily exposed residents of Seveso (*9,21*). They took into account the differences in dose and timing between the residents and the monkeys in the study by Rier et al. (*9*). In all cases the estimates from the area under the time–concentration curve exceeded the values for the monkeys receiving the higher dose (25 ppt). Thus, if humans are as sensitive as rhesus monkeys, the TCDD exposure levels in the Seveso cohort should be sufficient to result in endometriosis (*21*).

Twenty years after the accident, we conducted the Seveso Women's Health Study (SWHS). The primary goal was to determine if there was an association between TCDD exposure and endometriosis among women of reproductive age who had resided in the exposed area. Serum samples collected soon after the accident and analyzed for this study made it possible to quantify individual TCDD levels (*22*).

Methods

The Seveso accident. About noon on 10 July 1976, an explosion occurred in the ICMESA chemical plant near Seveso, Italy, approximately 25 km north of Milan. Up to 30 kg of TCDD were deposited over an area of about 18 km^2 (*23*). The area around the plant site was divided into zones based on soil levels of TCDD. Zone A, the most heavily contaminated area, housed 736 residents, all of whom were evacuated within 2 weeks after the accident. Zone B, the area of next greatest contamination, housed almost 4,500 residents who were not evacuated but warned about consuming locally grown food. Zone R, the least contaminated area, housed about 35,000 residents who were neither warned nor evacuated (*24*). A nonexposed area was delineated as zone non-ABR and encompassed the surrounding region of 180,000 inhabitants. As part of a health assessment, a blood sample was collected soon after the explosion for clinical chemistry tests; the remaining portion of the serum was stored for future studies (*20*). Initial analyses of this population revealed that the population was highly exposed to TCDD, but not to PCDDs or PCDFs (*22*); PCB congeners were not measured.

Study population. Women eligible for SWHS were 30 years old or younger in 1976, had adequate stored sera that had been collected between 1976 and 1980, and had resided at the time of the accident in zones A or B. A total of 953 women met these criteria. Twelve women could not be located or reached, seven had died, and nine were too ill to participate. Of the remaining 925 women, 751 (81%) agreed to participate. We excluded 54 virgins, 3 women with Turner's syndrome, and 93 women who refused the examination or ultrasound. This left 601 participants, who were similar to the eligible women in age and zone of residence.

Procedure. Study participation included obtaining informed consent, drawing blood, conducting a detailed interview, and performing a gynecologic examination and a transvaginal ultrasound. Each woman was interviewed by a trained nurse-interviewer, who was blinded to the woman's serum TCDD level and zone of residence. The structured interview covered sociodemographic information, personal habits, and work and medical history. The women were specifically asked whether they had experienced pelvic pain other than during their periods, deep pain with intercourse (dyspareunia), and menstrual cramps (dysmenorrhea). They were asked to rate the pain as mild, moderate, or severe with operational definitions provided as below:

- Pelvic pain: mild—occasional pelvic discomfort; moderate—noticeable discomfort for most of the cycle; severe—requires strong analgesics, persistent pain during cycle other than during menstruation.
- Dyspareunia: mild—tolerated discomfort; moderate—intercourse painful to the point of causing interruption; severe—avoids intercourse because of pain.
- Dysmenorrhea: mild—some loss of work efficiency; moderate—in bed part of the day, occasional loss of work; severe—in bed one or more days, incapacitation.

Women were also asked to rate the level of dysmenorrhea, pelvic pain, or dyspareunia on a 10-cm line, where the left end indicated "no pain" and the right end indicated "unbearable pain." In addition, women were asked whether they had ever tried for a year or more to get pregnant; that is, whether they did not do anything to prevent pregnancy for a year or more and did not get pregnant (infertility). We gathered detailed information about their gynecologic and reproductive histories. Medical records were requested for all gynecologic conditions, diseases, or procedures as well as for chronic diseases. Medical records were abstracted onto a form and coded by *International Classification of Diseases* (*25*) codes by a gynecologic nurse.

Gynecologists at the University of Milan, Mangiagalli Hospital, and at the Desio Hospital conducted the examinations. The ultrasounds were conducted both abdominally and transvaginally. Ultrasounds were recorded on videotape, and photographs were taken of ovaries and of any pathologic tissue.

Diagnostic process. The only definitive method for diagnosis of endometriosis is abdominal surgery. We conducted a validation study in a clinic-based population in parallel with this study that indicated that symptoms or signs were not good predictors of disease, but that ultrasound had excellent specificity and sensitivity for ovarian endometriosis (*26*). Thus, a woman was considered a "case" only if she had endometriosis noted on a laparoscopy or laparotomy or if she had a positive ultrasound (one in which a cyst or mass characteristic of endometriosis was noted, i.e., thick walls, regular margins, and homogeneous low echogenicity of fluid) (*27*).

Before surgical findings, findings on ultrasound and signs and symptoms were used to divide the remaining women into two groups: "nondiseased" or "uncertain" for disease status. A woman was considered nondiseased if she had surgery without a finding of endometriosis or if she had a negative ultrasound, exam, and symptom history. A woman who had surgery in the past (after 1976) could be considered nondiseased if she had no report of endometriosis at surgery, no subsequent increase in intensity of symptoms in the years after surgery, and no physical signs at the study's examination that would indicate the development of endometriosis after surgery. Signs of a positive exam included painful nodules, uterosacral ligament scarring, pain at the pouch of Douglas, Douglas nodularity, vaginal lesions/endometriotic lesions, painful/fixed adnexal masses, or fixed uterus. A positive symptom history included a report of infertility, a verbal report of moderate or severe pelvic pain, dysmenorrhea, or dyspareunia; or a pain rating in the right half of a 10-cm line for dysmenorrhea, pelvic pain, or dyspareunia. A woman was considered uncertain for disease if she had no surgery and a negative ultrasound, but she had signs on exam and/or reported symptoms. In some statistical analyses, women with only signs or symptoms were considered nondiseased based on the validation study that suggested that only a small percentage of

these women would have endometriosis at laparoscopy (*26*).

If any abnormality was noted on ultrasound, repeat ultrasound was offered. Laparoscopy was offered to women who had an ovarian cyst or mass noted on ultrasound; current severe dysmenorrhea, pelvic pain, or dyspareunia; or unexplained infertility in women younger than 40 years old. All laparoscopies were videotaped; lesions were excised, if possible, and sent for histologic evaluation.

The examining gynecologist assigned disease status. The consultant gynecologist independently reviewed the study materials. Those responsible for diagnosis were blind to the woman's exposure (zone or TCDD level).

Laboratory analyses. We preferentially selected the earliest serum sample available and sent the samples from Desio to the U.S. Centers for Disease Control and Prevention (CDC) for TCDD analysis by high-resolution mass spectrometry methods (*28*). Values were reported on a lipid-weight basis in parts per trillion (*29*).

TCDD was measured in sera collected between 1976 and 1977 for 559 women (93%), between 1978 and 1981 for 25 women (4%), and in 1996 for 17 women (3%) whose earlier samples had insufficient volume. For women with post-1977 TCDD values that were detectable but ≤ 10 ppt (n = 4), the measured value was used. For women with post-1977 TCDD levels > 10 ppt, the TCDD exposure level was back-extrapolated to 1976 using the Filser model (*30*) for women ≤ 16 years old in 1976 (n = 16) and using a first-order kinetic model for older women (n = 14) (*31*). For nondetectable values (n = 77), one-half the detection limit was assigned (*32*). For the study median serum sample weight of 0.65 g, the median limit of detection was 18.8 ppt, lipid adjusted. Because of the small volume of serum, it was not possible to measure other PCDDs, PCDFs, or PCBs and to still maintain a relatively low limit of detection for TCDD, the known exposure.

Statistical analyses. We modeled serum TCDD both as a continuous (log TCDD) and a categorical variable. We chose categories of ≤ 20.0 ppt, 20.1–00 ppt, and > 100 ppt. We chose 20 ppt (body burden ~4 ng/kg) as the lower limit, because the median value was between 15 and 20 ppt for 11 pooled serum samples collected from women residing in an unexposed area (zone non-ABR) at the time of the accident (*33*). We chose the 100 ppt limit (body burden

Table 1. Serum TCDD levels and endometriosis disease status by select characteristics of study participants, SWHS, Italy 1996–1998.

Characteristics	Median TCDD (Q_1–Q_3)[a] (ppt)	No. (%)			
		Total	Cases	Uncertain	Nondiseased
Zone of residence at accident					
A	257.0 (114.0–713.0)	97 (16)	2 (2)	50 (52)	45 (46)
B	47.0 (22.5–220.0)	504 (84)	17 (3)	255 (51)	232 (46)
Age at follow-up (years)					
20–29	166.5 (56.2–286.5)	120 (20)	5 (4)	67 (56)	48 (40)
30–39	52.2 (24.4–121.5)	236 (39)	4 (2)	126 (53)	106 (45)
≥ 40	44.0 (22.0–92.2)	245 (41)	10 (4)	112 (46)	123 (50)
Education					
< Elementary	43.7 (22.0–105.0)	141 (23)	6 (4)	60 (43)	75 (53)
Required	54.3 (24.4–131.0)	135 (22)	3 (2)	72 (53)	60 (44)
Intermediate professional	62.8 (33.2–171.0)	197 (33)	7 (4)	102 (52)	88 (45)
> Secondary school	59.0 (26.6–207.5)	128 (21)	3 (2)	71 (55)	54 (42)
Marital status					
Never married	148.0 (46.9–268.0)	95 (16)	2 (2)	52 (55)	41 (43)
Ever married	49.5 (23.5–123.0)	506 (84)	17 (3)	253 (50)	236 (47)
Current employment					
Employed	61.3 (26.2–172.0)	412 (69)	13 (3)	209 (51)	190 (46)
Not employed	48.0 (25.4–123.0)	173 (29)	5 (3)	85 (49)	83 (48)
Cigarette smoking					
Never	56.8 (25.6–157.5)	352 (59)	13 (4)	175 (50)	164 (47)
Former	51.8 (28.4–121.0)	101 (17)	4 (4)	53 (52)	44 (44)
Current	55.1 (26.0–170.5)	148 (25)	2 (1)	77 (52)	69 (47)
Alcohol consumption					
Never	54.2 (25.9–156.0)	399 (66)	14 (4)	214 (54)	171 (43)
Former	28.3 (12.5–84.7)	28 (5)	2 (7)	15 (54)	11 (39)
Current	61.0 (31.4–182.0)	174 (29)	3 (1)	76 (44)	95 (55)
Current body mass index (kg/m^2)					
≤ 20	102.0 (42.1–217.0)	104 (17)	4 (4)	57 (55)	43 (41)
20–25	54.8 (26.8–154.5)	340 (57)	11 (3)	168 (49)	161 (47)
≥ 25	43.2 (19.5–107.0)	157 (26)	4 (3)	80 (51)	73 (47)
Gravidity					
0	152.5 (49.0–272.0)	142 (24)	6 (4)	72 (51)	64 (45)
1–2	50.0 (24.8–119.5)	308 (51)	10 (3)	163 (53)	135 (44)
≥ 3	40.7 (21.6–86.2)	151 (25)	3 (2)	70 (46)	78 (52)
Oral contraceptive use (total years)					
0	48.2 (24.2–134.5)	208 (35)	4 (2)	87 (42)	117 (56)
< 2	55.2 (24.4–158.0)	169 (28)	7 (4)	98 (58)	64 (38)
3–5	64.6 (29.2–159.0)	106 (18)	4 (4)	55 (52)	47 (44)
> 5	65.0 (37.0–190.0)	116 (19)	3 (3)	65 (56)	48 (41)
Menarche status at accident					
Premenarche	130.5 (46.9–251.0)	190 (32)	6 (3)	105 (55)	79 (42)
Postmenarche	44.4 (22.0–96.2)	411 (68)	13 (3)	200 (49)	198 (48)
Chloracne					
Yes	1575.0 (168.0–3180.0)	18 (3)	1 (5)	10 (56)	7 (39)
No	53.7 (25.0–142.0)	583 (97)	18 (3)	295 (51)	270 (46)

[a] Q_1 = 25th percentile; Q_3 = 75th percentile.

~20 ng/kg) because previous studies in Seveso reported an effect at about this level (*34*).

We performed statistical analyses using STATA 6.0 (*35*). We performed polytomous logistic regression analysis using endometriosis as the outcome with categories "cases," "uncertain," and "nondiseased." Effects were characterized by relative risk ratios (RRRs). We also present crude and adjusted percentages. The form of the dose–response curve for the probability that a woman had endometriosis was investigated using fractional polynomials in logistic regression (*36*).

Potential confounders and effect modifiers were selected from the literature (*8*) and included the variables presented in Table 1. None confounded (changed the TCDD parameter estimate by more than 10%) or modified the TCDD–endometriosis association. Only age was retained in the final models.

Results

We identified 19 cases out of 601 women (3.2%): 14 with a surgically confirmed diagnosis of endometriosis and five who refused laparoscopy but who had ovarian endometriosis diagnosed by ultrasound. A total of 277 women were classified as nondiseased: 238 had neither signs (on examination) nor symptoms (pain or infertility) of endometriosis, and 39 had no endometriosis found at pelvic surgery performed either during this study or in the past. The remaining 305 women were uncertain for endometriosis, with 273 of these women having either signs or symptoms.

Table 1 displays the relation of various potential covariates with exposure and disease status. Most women (80%) were ≥ 30 years of age at follow-up (at least 10 years of age in 1976); approximately two-thirds of the women in the cohort were postmenarcheal at the time of the accident. Serum TCDD levels tended to be higher in the women who were < 10 years old at the time of the accident and in women who lived in zone A. Higher levels were also found in women who were premenarcheal at the time of the accident, never married, nulligravid, and of lower current body mass; however, these women were also younger.

The distribution of TCDD for all women and for women classified by disease status is presented in Figure 1. The overall median serum TCDD level was 54.9 ppt with a range of 2.5–17,300 ppt. The median serum TCDD level for cases was 77.3 ppt; for nondiseased was 61.0 ppt; and for the uncertain group was 49.0 ppt. The TCDD levels for cases and nondiseased overlapped at the tails of the cumulative distribution, but cases had higher TCDD levels in the middle of the distribution. The uncertain group (mean = 36.1 years, SD = 7.9) was younger than cases (mean = 37.6 years, SD = 8.2) and the nondiseased group (mean = 37.8 years, SD = 8.1; $p = 0.04$).

Table 2 shows the crude and age-adjusted frequencies of disease category by TCDD exposure. The adjusted percentages are the rates predicted by polytomous regression for a woman with the mean age of 37 years. Although the adjusted percentage of cases increased from 1.7% for women with ≤ 20.0 ppt serum TCDD to 4.6% for those with > 100 ppt, the percentage of nondiseased women also increased with exposure levels. Compared to the lowest dose group, the RRR of the moderate dose group is 1.2 (90% CI = 0.3–4.5) and the RRR of the highest dose group is 2.1 (90% CI = 0.5–8.0). The test for trend for the cases-to-nondiseased ratio (scoring categories as 1, 2, 3) was nonsignificant ($p = 0.25$). The test for trend with continuous log TCDD in the polytomous model was also nonsignificant ($p = 0.84$). Fractional polynomials in logistic regression showed no indication of increasing relative risk with log TCDD.

When we moved the women with either signs or symptoms to the nondiseased group, the RRRs for cases relative to the new nondiseased group were 1.6 (90% CI, 0.4–5.9) for the 20.1–100 ppt group and 2.8 (90% CI, 0.7–10.3) for the > 100 ppt group. The RRRs for the uncertain group

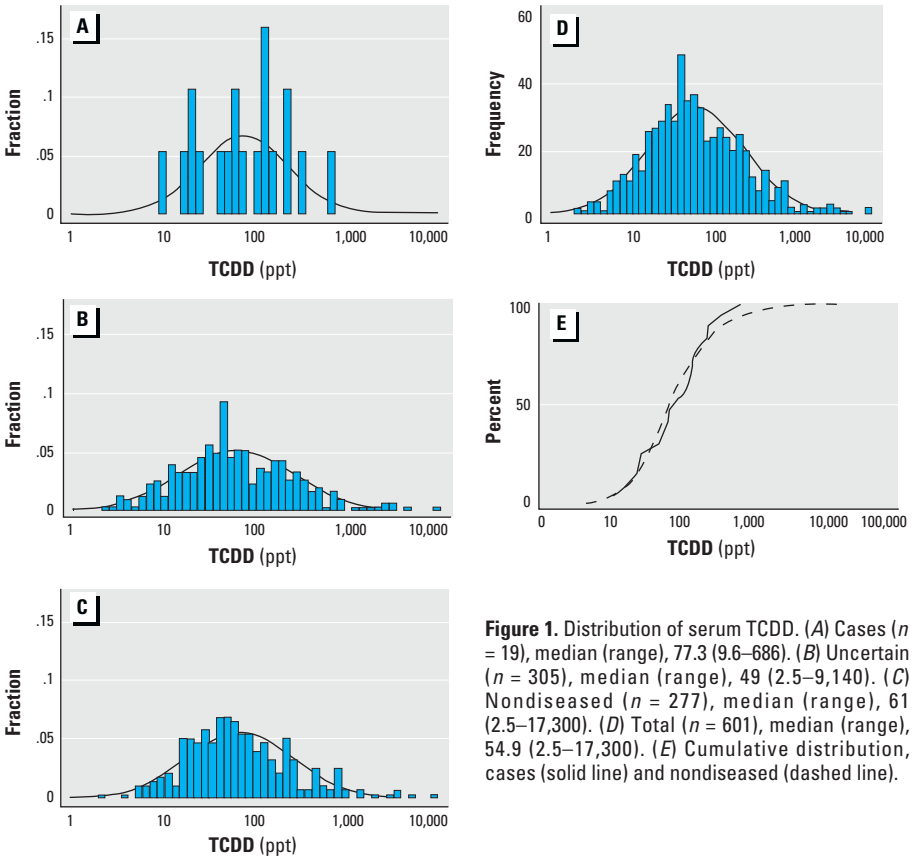

Figure 1. Distribution of serum TCDD. (*A*) Cases (n = 19), median (range), 77.3 (9.6–686). (*B*) Uncertain (n = 305), median (range), 49 (2.5–9,140). (*C*) Nondiseased (n = 277), median (range), 61 (2.5–17,300). (*D*) Total (n = 601), median (range), 54.9 (2.5–17,300). (*E*) Cumulative distribution, cases (solid line) and nondiseased (dashed line).

Table 2. Crude and age-adjusted frequencies and age-adjusted RRR of TCDD exposure and endometriosis disease classification.

Disease classification	TCDD (ppt) ≤ 20.0 (n = 111)	20.1–100 (n = 285)	> 100 (n = 205)
Cases			
Unadjusted frequency (n)	1.8% (2)	2.8% (8)	4.4% (9)
Adjusted frequency	1.7%	2.7%	4.6%
RRR (90% CI)	1.0 (referent)	1.2 (0.3–4.5)	2.1 (0.5–8.0)
Uncertain			
Unadjusted frequency (n)	60.4% (67)	47.4% (135)	50.2% (103)
Adjusted frequency	61.8%	48.3%	48.0%
RRR (90% CI)	1.0 (referent)	0.6 (0.4–0.9)	0.6 (0.4–0.9)
Nondiseased			
Unadjusted frequency (n)	37.8% (42)	49.8% (142)	45.4% (93)
Adjusted frequency	36.5%	49.0%	47.3%
RRR (90% CI)	1.0 (referent)	1.0 (referent)	1.0 (referent)

were now closer to 1.0. The tests for trend remained nonsignificant (p = 0.15 for TCDD categories; p = 0.55 for continuous log TCDD).

When we compared the cases with only the portion of the nondiseased group who had had pelvic surgery (n = 39), the RRRs were 0.7 (90% CI, 0.2–3.4) for the 20.1–100 ppt group and 2.3 (90% CI, 0.4–11.3) for the > 100 ppt group. The tests for trend remained nonsignificant (p = 0.29 for TCDD categories; p = 0.99 for continuous log TCDD). In addition, when we compared only the 14 surgically confirmed cases with the 39 surgically confirmed nondiseased, the RRRs were 1.1 (90% CI = 0.2–7.8) for the 20.1–100 ppt group and 3.6 (90% CI = 0.5–27.0) for the >100 ppt group. The tests for trend remained nonsignificant (p = 0.14 for TCDD categories; p = 0.83 for continuous log TCDD).

Discussion

The Seveso Women's Health Study is the first study to investigate the relation between TCDD exposure and endometriosis in a large population of women with a wide range of exposure. We found that women with serum TCDD levels of 100 ppt or higher had a doubled but statistically nonsignificant risk for endometriosis. There was also no evidence of a dose–response relationship. Our results are consistent with the nonsignificant odds ratio of 4.3 associated with serum levels > 100 ppt TEQ recently reported in a Belgian case–control study of infertile women (*17*).

Our study has some important limitations, the most notable of which were the limited power due to the small number of women with endometriosis and our inability to perform laparoscopy on every woman and thereby to determine definitively the disease status for the entire cohort. We conservatively chose to identify cases based only on women who had endometriosis identified by surgery or ultrasound. Although 34 women were offered laparoscopy during the study based on appropriate medical criteria, only nine accepted. Therefore, we may have missed cases of endometriosis among the portion of women in the uncertain or nondiseased groups who had not had surgery or ovarian endometriosis (diagnosable by ultrasound). This misclassification would lead to an underestimate of the risk, given that it is unlikely that there was differential disease misclassification; neither the investigators who made the diagnoses nor the interviewers or respondents knew the TCDD levels, the CDC laboratory had no information about disease, and the interviewers and respondents were unaware of study hypotheses.

This study may have underestimated the effects of TCDD in that the group that was most heavily exposed, the youngest women, may have been underrepresented. For example, for cultural reasons we were unable to examine women who had never been sexually active, and they were more likely to be younger. In addition, because endometriosis appears during the reproductive years, younger women would have less of an opportunity for disease diagnosis. Although we controlled for age in the analysis, the possibility of residual confounding by age remains.

One of the strengths of this study is that use of sera stored from the time of the accident allowed us to have a direct measure of an individual's TCDD level to correlate with the disease end point. As part of the SWHS, we were able to analyze these sera for TCDD for the first time on a large segment of the Seveso cohort. We chose as our definition of low exposure ≤ 20 ppt, based on values from pooled samples taken from women living in an unexposed area of Italy in 1976. These serum levels are higher than current background levels (< 10 ppt) (*37*). We did not measure levels of other dioxin-like compounds (PCDDs, PCDFs, PCBs) that might have resulted from background exposure in 1976. Because the exposure from the Seveso explosion was specific to TCDD, the TEQ in this cohort is likely be dominated by their exposure to TCDD. However, if the cohort had substantial levels of other dioxin-like compounds due to background exposure and the potential effect derived from total TEQ, the relative difference across TCDD exposure groups would have been attenuated. Nevertheless, a larger number of women with less exposure would not have changed our conclusion, given that the prevalence of endometriosis in the ≤ 20 ppt group was already very low (1.8%).

In summary, we found a doubled, nonsignificant, risk for endometriosis among women with serum TCDD levels of ≥ 100 ppt. To eliminate the possibility of exposure misclassification, future studies should determine whether there was substantial exposure to other PCDDs, PCDFs, and dioxin-like PCBs in this population. Finally, a more definitive study of endometriosis in the Seveso cohort should be conducted when a noninvasive biomarker for endometriosis is developed that can use the blood samples recently collected from this cohort. This would reduce the potential disease misclassification inherent in a population-based cohort study of endometriosis.

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